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Marking Method for Sea Urchin *Strongylocentrotus intermedius* with Brilliant Red Dye

(Buririanto Reddo ni yoru Ezobafun Uni no Hyoshiki-ho)

by Ken'ichiro TAJIMA (Fisheries Experimental Station of Wakkanai)

in Hokusuishi Geppo (abbreviated title; Monthly Bulletin of the Hokkaido Fisheries Experimental Station) 1982, Vol.39, No. 4, April, pp. 81 - 87 (Art. No. B 1817)

To investigate the effect of transplanting or releasing sea urchins, or their migrations, growth and such, one often attaches tags to specimens. Up to now, one made a hole in the shell of the sea urchin, and passed through the hole a nylon thread or such other similar item [1) - 3)]. One also tried attaching a rubber band [1)], driving a nylon tag into the shell [4)] and some other similar methods. One also tried marking by dyeing live specimens, and it was reported that nile blue was effective with adult and juvenile *Strongylocentrotus intermedius* (abbreviated hereafter as Si : translator) sea urchins [2) 5)]. It was also reported that brilliant red was more effective than nile blue with regard to Bafun sea urchins [6)] *.

In order to check whether brilliant red which is effective with Bafun sea urchins is also effective with Si sea urchins, we performed tests, changing the concentrations and dyeing times, and examined the dye conditions,

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* Translator's note : *Strongylocentrotus pulcherrimus* auct.

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vigor recovery, growths and deaths. We also conducted a survey, releasing dyed sea urchins. We present hereunder some information we obtained.

Method

We started our tests at the Wakkanai Fisheries Laboratory from November 10, 1979, using Si sea urchins from the Rebun island. We used as samples sea urchins with shell diameters of 20 - 47 mm, and weights of 3.0 - 43.9 g. To dye, we dissolved Mication (?) Brilliant Red 8 BS from the Mitsubishi Chemical Industries, Ltd. (abbreviated hereafter as brilliant red) at the concentrations of 2 % and 1 % in seawater, and stirred thoroughly. The dyeing times for each concentration were 60, 30 and 10 min. Together with the controls, there were thus seven groups. One group consisted of 18 - 19 individuals. Air was circulated during the dyeing operation, and the water temperature was kept at about 16 ºC. After the dyeing, we washed several times with seawater so as to remove the excess dye solution, after which we put the specimens in a rearing tank (45 x 35 x 30 cm). We divided the dyeing

p. 82

conditions into the following 5 stages on the basis of the 4-stage table of Kawamura et al [5].

A : The whole body is deeply dyed

B : The whole body is lightly dyed

C : Part of the body (spines, tube feet and such) is dyed

D : Part of the body is very lightly dyed

E : The body is not dyed

We observed the vigor recovery on the basis of the motility of the tube feet 5 min, 10 min, 30 min, 60 min, 120 min, 180 min after the dyeing. Furthermore, in order to observe the growths, we measured the body weights prior to dyeing, and on the 40th, 80th, 133rd and 174th days after dyeing, and observed the dye conditions as well. During the rearing period, the water temperature was 0.2 - 9.3 ºC. For food we provided plenty of Rishiri kelp. When it was not possible to obtain Rishiri kelp on account of the sea being frozen, we provided dried Rishiri kelp.

Two days after they had been dyed at 2 % for 30 min on October 2, 1980, we released the Si sea urchins in the waters of Wakkanai city. On May 28 and October 7, 1981, we recaptured some of the specimens and observed their dye conditions.

Results and discussion

1. Dye conditions

The sea urchins dyed with brilliant red were dyed red on their tube feet, peristome, shell, top part and such. Table 1 shows the time-course changes in the dye conditions according to the dye concentration and the dyeing time.

In the two 2 % - 60 min and 1 % - 60 min groups, almost all individuals were deeply dyed over the entire body immediately after the procedure. On the 40th day, we noted sone fading, and the dye was light over the entire body. Up to the 133rd day, we noted no great changes. On the 174th day, we noted some fading in the 1 % - 60 min group.

In the 2 % - 30 min group, there were immediately after the operation some individuals that were deeply dyed as well as some others that were only lightly dyed over the entire body. The respective proportions were 47 % and 53 %. On the 40th day, there were individuals with an entire lightly dyed body,

and some others with only a partly dyed body, the proportions being 63 % and 37 %. On the 80th day, the respective proportions were 26 % and 74 %. Thereafter, up to the 174th day, we noted no great changes.

In the two 1 % - 30 min and 2 % - 10 min groups, immediately after the dyeing, there were already no individuals with a whole deeply dyed body. In the former group there was 53 % of individuals with lightly dyed bodies, 42 % with partly dyed bodies, and 5 % with partly and faintly dyed bodies. In the latter group, there was 56 % of individuals with partly dyed bodies, and 44 % of individuals with partly and faintly dyed bodies. Even immediately after the dyeing, the dye conditions were poor. In both groups, most individuals had on the 80th day only partly and faintly dyed bodies. Although fading progressed up to the 174th day, it was still possible to distinguish the marked from the unmarked specimens.

In the 1 % - 10 min group, only part of the body was faintly dyed immediately after the dyeing, and on the 40th day, 44 % of the individuals could not be distinguised any more. On the 174th day, 67 % of the individuals could not be distinguished any more.

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When using brilliant red with Bafun sea urchins, Tsunoda et al [6] reported that if one dyes at the 0.5 %, 1 %, 2 % concentrations for the length of time of 30 min, all individuals showed good dye conditions even after 4

p. 84

months, that if one dyed at 2 % for 60 min, almost no fading trend could be noted, and that even after one year, one could still recognize easily the dyed individuals. In the case of Si sea urchins dyed at 2 % - 60 min, 1 % - 60 min, 2 % - 30 min, discrimination was still easy after about a half year. Yet, immediately after dyeing at 1 % - 30 min, the dye condition was already poor. If we thus compare the dye conditions 30 min after the dyeing, we see that the dye of the Si sea urchins dyed at 1 % was bad whereas that of Bafun sea urchins dyed at 0.5 % was still good after 4 months. There seems to be differences in the ways of becoming dyed depending on the species.

2. Vigor recovery

Because of the dyeing, the vigor of the sea urchins declined, the tube feet shrank and movements stopped for a while. Fig. 2 shows the vigor recovery on the basis of tube feet movements.

For the 10 min following dyeing, we noted no movement in the 2 % - 60 min group. After 30 min 5 % of the individuals, and after 180 min 63 % of the individuals moved their tube feet, and we observed that vigor was restored as time went by. However, it was only after 48 h that all individuals were seen to move their tube feet. In the 1 % - 60 min group, 10 % of the individuals were seen moving their tube feet after 30 min, 58 % after 60 min, and 89 % after 180 min. We had to wait 48 h to see all individuals moving actively their tube feet.

Meanwhile, in the 2 % - 30 min group, 68 % of the individuals were observed to move their tube feet after 5 min, and 89 % after 10 min. The tube feet of all individuals were moving after 30 min. Recovery was noted to be fairly faster than in the two former groups. In all three 1 % - 30 min, 2 % - 10 min and 1 % - 10 min groups, all individuals were observed to move their tube feet after 5 min.

p. 85

The physiological effect of dyeing Si sea urchins with nile blue comes to an end within a short time in the case of adults. With juveniles, the

recovery of vigor takes 24 h [2)5)]. In the two 2 % brilliant red - 60 min and 1 % - 60 min groups, vigor recovery required a long time, and the physiological effect seemed to have been serious. In the other four groups, the tube feet began to move 5 - 30 min after the dyeing. Vigor recovery seemed to be faster and the physiological effect rather weaker than in the two above groups.

Growth

Table 3 shows the growth of 1 individual per day for each test group.

During the 40 days from November 10 to December 20, the growths were only 10 mg and 4 mg respectively for the two 2 % - 60 min and 1 % - 60 min groups, but for the other test groups, it was 45 mg - 75 mg, that is, remarkably less than the 105 mg noted in the control group. During the 40 days from December 20 to January 29, the growths were 19 mg and 10 mg for the two 2 % - 60 min and 1 % - 60 min groups, which is 32 - 51 mg less than in the control group. Yet, from the 40th day following dyeing, we noted an increasing tendency. From January 29, no extreme values were observed in spite of some scattering in the growths.

Kawamura et al [5)] reported that when dyeing juvenile Si sea urchins with nile blue, growth was still poor three months later with regard to almost all individuals that had been more deeply dyed. In the present tests too the

p. 86

deeply dyed groups tended to grow poorly until the 80th day after the dyeing, but such tendency was definitely absent from the 80th day. It is thus estimated that the growth impediment caused by such dyeing is gradually alleviated after a given period of time.

4. Deaths

We observed 1 dead individual in the 1 % - 30 min group during the time from the start of the tests up to December 30, 2 dead individuals in the 1 % - 10 min group from December 20 to January 29 and 1 dead individual in the 1 % - 60 min group from March 22 to the end of the tests. Kawamura - Hayashi [2)] reported that there were practically no deaths when dyeing Si sea urchins with nile blue, and Tsunoda et al [6)] reported that no deaths had been observed when dyeing Si sea urchins with brilliant red. Deaths were observed in the present tests, but because of the season it does not seem that such deaths were directly caused by the dyeing. Be it what it may, it is believed that if the

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dyeing concentration, time and temperature are appropriate for live sea urchins, normally the dyeing should not bring about death.

Marked release

On October 4, 1980 we released in natural sea waters 937 Si sea urchins which had been dyed at 2 % for 30 min, the dyeing conditions being those judged in indoor tests to be effective for marking. On May 28 (the 236th day) and on October 7, 1981 (the 368th day) we recaptured part of the released specimens and observed the dye conditions. On the 236th day, 60 % of the specimens were still lightly dyed on the whole body and 40 % still partly dyed. On the 368th day, 67 % of the individuals were still lightly dyed on the whole body, and 33 % only partly dyed.

We were able to recapture only part of the individuals released, so we do not know the survival rate. Yet, there were no faintly and partly dyed specimens among the individuals we recaptured, and the marks could in all cases be recognized easily. The color was however a little faded, as compared to the results of the indoor tests.

The above results suggest that brilliant red provides effective marking for Si sea urchins, and that it is possible to achieve an approximate one-year marking by using this dye at the water temperature of 16 °C, and at the 2 % concentration, with a dyeing time of 30 min.

There are however limitations. The meaning of the dye-marking method would cease to exist if the dye mark came to fade and if the individuals could

p. 87

not be distinguished any more. It would also be difficult to use this method with murasaki sea urchins * as they have already of a deep purple color. The next task will be to develop a dyeing method that prevents fading and that can be used for a long period of time, and to develop a technique allowing to attach individual identification marks safely and rapidly.

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* Translator's note : *Heliocidaris crassispina* (A. agassiz) auct.

Summary

We dyed live Si sea urchins with brilliant red and obtained the following results.

1. The Si sea urchins dyed at 2 % - 60 min, 1 % - 60 min and 2 % - 30 min with a 16 °C water temperature were well dyed, and could be recognized easily after 174 days.

2. Dyeing at 2 % - 60 min, 1 % - 60 min lowers temporarily and markedly the vigor of the specimens, and about 2 days are required for a recovery.

3. The growth impediment caused by 2 % - 60 min and 1 % - 60 min dyeing was pronounced for 80 days, but unclear thereafter.

4. At a concentration not higher than 2 %, there were no deaths immediately after the dyeing when the dyeing time did not exceed 60 min. There were few deaths during the following rearing.

5. It was possible for one year to recognize released individuals that had been dyed at 2 % - 30 min.

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Graphs and Tables

Table 1 Time-course changes in the dye condition of Si sea urchins

a. concentration, b. dyeing time (min), c. dyeing stage, d. number of days, e. --th day

A entire body deeply dyed. B entire body lightly dyed. C body partially dyed. D body partially and faintly dyed. E body not dyed

Table 2 Proportion of individuals showing no tube feet motion after dyeing

a. concentration, b. dyeing time (min), c. time, d. minutes, e. hours

Table 3 Time-course changes in the growth (mg/individual/day) of dyed Si sea urchins

a. concentration, b. dyeing time (min), c. time, d. --th month, e. --th day, f. control g. average amount of growth

第1表　エゾバフンウニの染色状況の経時変化　Table 1

a 濃度 %	b 染色時間 分	c 染色段階 ＼ 日数 d	e 0日目	e 40日目	e 80日目	e 133日目	e 174日目
2	60	A	100%				
		B		100%	100%	100%	100%
	30	A	47				
		B	53	63	26	26	21
		C		37	74	74	79
	10	C	56	56	6	6	
		D	44	44	94	94	100
1	60	A	89				
		B	11	100	100	100	83
		C					17
	30	B	53				
		C	42	67	11		
		D	5	33	89	100	100
	10	D	100	56	56	56	33
		E		44	44	44	67

A：体全体が濃く染まる。B：体全体が薄く染まる。C：体の一部が染まる。
D：体の一部がわずかに染まる。E：体は染まらない。

Table 2　第2表　染色後に管足の運動が見られた個体の割合

a 濃度 %	b 染色時間 分 ＼ 時間 c	d 5分	d 10分	d 30分	d 60分	d 120分	d 180分	e 48時間
2	60	0%	0%	5%	10%	47%	68%	100%
	30	68	89	100				
	10	100						
1	60	0	0	10	58	79	89	100
	30	100						
	10	100						

Table 3　第3表　染色したエゾバフンウニの成長量（mg/個体/日）の経時変化

a 濃度 %	b 染色時間 分	平均重量 g	期間 ＼ d	d 11月10日e ～ 12月20日e d	d 12月20日e ～ 1月29日e d	d 1月29日e ～ 3月22日e d	d 3月22日e ～ 5月2日e d
2	60	17.08		10	19	62	127
	30	18.16		45	35	60	89
	10	16.70		75	35	63	57
1	60	18.31		4	10	55	89
	30	19.77		73	32	78	110
	10	18.00		68	51	93	175
対照 f		18.92		105	39	44	123

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* Translator's note : reading uncertain; other readings such as "Kadota", "Kakuda" and such also possible